Dept. of Poultry Diseases,
Fac. Vet. Med. Assiut University

PREVALENCE OF AVIAN LEUKOSIS VIRUS IN CHICKEN FLOCKS IN UPPER EGYPT

(With 7 Tables)

By

S. MOUSA and M.H. ABDEL-WAHAB*

* Animal Production Research Institute. (Seds, Beni-Suef), Egypt

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دراسة مدي انتشار فيروس مرض الليوكوزس في قطعان الدجاج في صعيد مصر

صلاح موسي ، مختار عبدالوهاب

لدراسة مدي انتشار أنتيجن فيروس مرض الليوكوزس في مختلف قطعان الدجاج في صعيد مصر، تم تجميع عينات مصل وبيض من مختلف مزارع الدواجن والتي تمثل مختلف الأعمار ومختلف نظم التربية وأنواع الدجاج المختلفة والتي تشمل دجاج بيض المائدة، ودجاج التسمين وأمهات الدجاج اللاحم وبعض السلالات المحلية. وتم اختبار عينات المصل والبيض للكشف عن أنتيجن فيروس مرض الليوكوزس بواسطة اختبار الإليزا. أظهرت النتائج أن كافة قطعان الدجاج في صعيد مصر التي خضعت للإختبار احتوت علي فيروس مرض الليوكوزس بنسب متفاوتة. وكانت النسب الأعلي لتواجد الفيروس في بداري التسمين والسلالات البلدية. وكانت نسبة الفيروس الداخلي لمرض الليوكوزس أعلي من نسبة الفيروس الخارجي.

SUMMARY

To study the prevalence of ALV antigen in different poultry flocks in Upper Egypt, serum and egg samples were collected from poultry farms representing different ages, different raising systems and different chicken types including table egg layers, meat type broilers, breeders and random native breeds. Samples were tested for detection of P.27 Common antigen of ALV by ELISA test. All chickens flocks possessed ALV antigen at various degrees. The highest percentage was detected in meat type chicken flocks and balady chickens. Titre of endogenous virus was greater than exogenous virus.

Key words: *Leukosis, exogenous leukosis*

INTRODUCTION

Tumours continue to be a major cause of economic losses among poultry industry in many countries including Egypt. Economic impact includes mortalities, condemnations and immune suppression.

Avian Leukosis Viruses (ALV) known as avian retroviruses induce a variety of tumours (neoplasms) of which lymphoid leukosis is common. Based on the discovery of the importance of infection at embryonic stage, extensive control programmes were initiated to control the spread of ALV (Arshad *et al.,* 1997a). Vertically infected chicks (at embryonic stage) and chicks horizontally infected (at 1-day-old stage or during the rearing period) are at risk to become carriers of ALV. These are able to shed large amounts of the ALV into the environment and form a source of infection to uninfected hatchmates. Infected birds are likely to develop tumours and a high percentage of laying hens among these birds will in turn transmit virus vertically. Thus, culling of hens that are viraemic and shed virus in eggs will greatly reduce the vertical transmission of ALV (Arshad *et al.,* 1997b).

The presence of virus is determined by the detection of ALV P27 by indirect biologic assays, such as complement fixation (CF) for avian leukosis (COFAL) (Sarma *et al.,* 1964). ELISA for ALV (Crittenden *et al.,* 1987; Fadly and Witter, 1998) phenotypic mixing (Okazaki *et al.,* 1975) resistance inducing factor (Rubin, 1960) and nonproducer cell activation (Rispens, 1970). Of all such assays, ELISA-AVL is the most commonly used test.

This work was planned to investigate the prevalence of tumours among different chicken types and ages through the determination of ALV antigen in chicken sera and albumen by ELISA test.

MATERIALS and METHODS

- **Sera**: Blood samples were collected from layers, breeders, balady and broiler flocks from different areas in Upper Egypt. Sera were subjected to ELISA test for detection of LAV antigen.
- **Egg albumen**: Eggs were collected from different areas in Upper Egypt. Albumen was withdrawn by punching a small hole midway between the middle and the small end of the eggs, care was taken to avoid obtaining yolk materials. The albumen was assayed for ALV by ELISA test.

ELISA test:

- **Reagents:**

(a) ALV positive control

(b) Negative virus control

(c) Rabbit anti-p27 peroxidase conjugate (d) Dilution buffer
(e) ABTs-hydrogen peroxidase substrate (f) Stop solution
(g) Wash solution

- **ELISA reader:** 96 well plate reading ELISA reader with 405 nm filter. Reader was a "Biotek SL 311".
- **Preparation of controls:** A p27 positive and negative controls were provided with the kit. in a reading-to-use form. The p27 positive and negative control samples were allowed to equilibrate to room temperature.
- **Preparation of conjugate solution:** A horseradish peroxidase conjugated rabbit anti-P27 was supplied in 50% glycerol. 200 µl of the conjugate solution were diluted in 10 ml dilution buffer.
- **ELISA test procedure:** According to manufacture plates were processed as follows:
  (a) An anti-P27 antibody coated test plate was removed from the protective bag and labelled.
  (b) 100 µl negative control were directly added to wells As, H10 & H12 without dilution. Pipette tip was discarded each time.
  (c) 100 µl positive control was directly added to wells A1, A3 and H11 without dilution.
  (d) 100 µl of unknown sample (either albumen or serum were added to each well.
  (e) Plate was incubated for 30 minutes at room temperature.
  (f) Plates were washed.
  (g) Addition of anti-P27 peroxidase conjugate, substrate and solution.

Processing of DATA:

(a) Plates were read using an ELISA plate reader set at 405 nm and concentration of P27 antigen per sample was calculated by the following equation:

$$SP = \frac{\text{(Sample absorbance)} - \text{(average } - \text{ve control absorbance)}}{\text{corrected} + \text{ve control (CPC) absorbance}}$$

(b) By means of a software "proflock" produced by KPL USA, the titres standard deviations and coefficient of variation were calculated.

RESULTS

Results of ELISA test for detection of P-27 common ALV antigen are shown in tables (1-7). Tables (1-3) summarize the findings in correlation to age. It is clear that birds aged 3-5 months constituted the highest rate of positive cases (66.4%). On the other hand, tables (4-6) show the rate in correlation with chicken type. Layers showed the least rate (15.7%) while meat-type chickens, breeders and balady chickens showed higher rate (66.7% and 67.8%) respectively. For detection of exogenous virus disseminated in eggs, a total of 1311 samples were collected from eggs of different sources. Results illustrated in table 10 show that 32.3% of cases were positive for presence of exogenous virus.

Table 1: Results of detection of ALV antigen in sera of chickens up to 2 months of age

Source & chicken type	Count	ELISA titre					No. of positive samples	%
		Mean	GMT	CV%	Min.	Max.		
Menia layers	81	104	22	220.8	0	1046	16	19.7
Kena meat type	60	360	275	92.0	131	1003	26	43.3
Assiut breeders	49	127	49	121.9	0	512	17	34.6
Assiut balady	80	110	62	98.1	60	507	61	76.2
Total	270						120	44.4

Table 2: Results of detection of ALV antigen in sera of chickens of 3-5 months of age.

Source and chicken type	Count	ELISA titre					No. of positive samples	%
		Mean	GMT	CV%	Min.	Max.		
Assiut balady	90	111	9	270.4	0	2339	43	47.8
	90	160	33	137.2	0	973	65	72.2
	90	186	15	258.5	0	2698	49	54.4
	90	493	301	76.1	0	1842	88	97.8
	90	105	20	144.6	0	715	67	74.4
	90	75	11	204.4	0	823	55	61.1
	90	101	19	142.4	0	7.13	57	63.3
	90	90	20	120.0	0	507	58	64.4
Assiut breeders	49	546	488	53.5	186	1242	29	59.2
Total	760						511	66.4

Table 3: Results of detection of ALV antigen in sera of chickens more than 6 months of age.

Source and chicken type	Count	ELISA titre					No. of positive samples	%
		Mean	GMT	CV%	Min.	Max.		
Kena meat type	12	641	433	79.4	95	1688	12	100
	15	256	154	100.7	16	1039	15	100
	14	171	95	80.6	0	454	13	92.9
	15	193	95	98.3	0	581	14	93.3
Assiut breeders	90	427	127	116.5	0	2935	77	85.6
Menia layers	7	208	150	79.7	29	536	2	28.6
	8	121	52	108.4	0	362	2	25
	5	23	13	85.5	0	52	0	0
	7	180	50	152.0	0	760	3	42.9
	6	17	8	98.5	0	45	0	0
	7	40	14	107.8	0	110	1	14.3
	5	79	59	91.0	22	202	1	20
	9	82	13	222.4	0	562	3	
	6	30	7	157.2	0	123	1	16.7
	13	152	23	155.9	0	762	3	23.1
	5	38	9	145.4	0	1310	1	20
	2	37	9	141.4	0	73	0	0
	10	9	3	171.2	0	49	0	0
Assiut layers	90	79	9	196.1	0	599	8	8.9
Total	326						156	47.8

Table 4: Detection of ALV antigen in sera of layer flocks.

Source and chicken type	Count	No. of positive samples	%	ELISA titre				
				Mean	GMT	CV%	Min.	Max.
Assiut	90	8	8.9	79	9	19601	0	599
Minia	7	2	28.6	208	150	79.7	29	536
	8	2	25	121	52	108.4	0	362
	5	0	0	23	13	85.5	0	52
	7	3	42.91	180	50	152.0	0	760
	6	0	0	17	8	98.5	0	45
	7	1	14.3	40	14	107.8	0	110
	5	1	20	79	59	91.0	22	202
	9	3	33.3	82	13	222.4	0	562
	6	1	16.7	30	7	157.2	0	123
	13	3	23.1	152	23	155.9	0	762
	5	1	20	38	9	145.4	0	130
	2	0	0	37	9	141.4	0	73
	10	0	0	9	3	171.2	0	49
Menia	81	16	19.7	104	22	220.8	0	1046

Table 5: Detection of ALV antigen in sera of meat-type and breeder flocks.

Source and chicken type	Count	No. of positive samples	%	ELISA titre				
				Mean	GMT	CV%	Min.	Max.
Kena (meat type)	60	26	43.3	360	275	92.0	131	1003
	12	12	100	641	433	79.4	95	1688
	15	15	100	256	154	100.7	16	1039
	14	13	92.9	171	95	80.6	0	454
	15	14	93.3	193	95	98.3	0	581
Assiut (1)	49	17-	34.6	127	49	121.9	0	512
Assiut (2)	49	29	59.2	546	488	53.5	186	1242
Assiut (3)	90	77	85.6	427	127	116.5	0	2935
Total	304	203	66.7					

Table 6: Detection of ALV antigen in sera of balady chickens.

Source and chicken type	Count	No. of positive samples	%	ELISA titre				
				Mean	GMT	CV%	Min.	Max.
Assiut	80	61	76.2	110	62	98.1	60	507
Assiut	90	43	47.8	111	9	270.4	0	2339
	90	65	72.2	160	33	137.2	0	973
	90	49	54.4	186	15	258.5	0	2698
	90	88	97.8	493	301	76.1	0	1842
	90	67	74.4	105	20	144.6	0	715
	90	55	61.1	75	11	204.4	0	823
	90	57	63.3	101	19	142.4	0	713
	90	58	64.4	92	20	120.0	0	507
Total	800	543	67.8					

Table 7: Results of detection of exogenous ALV antigen in eggs (albumen):

Source and chicken type	Count	ELISA titre					No. of positive samples	%
		Mean	GMT	CV%	Min.	Max.		
Assiut breeders	39	54	5	277.7	0	691	17	43.6
Assiut layers 1	75	39	9	174.7	0	498	7	9.3
Assiut layers 2	90	43	4	220.0	0	476	8	8.9
Assiut layers 3	90	25	3	342.3	0	569	8	8.9
Menia layers 1	48	2	1	346.7	0	28	4	8.3
Menia layers 2	90	48	11	168.3	0	562	5	5.6
Menia layers 3	90	118	3	256.5	0	1498	9	10
Assiut Balady 1	30	22	5	223.3	0	256	14	46.7
Assiut Balady 2	42	66	13	148.8	0	418	25	59.5
Assiut Balady 3	90	411	17	231.8	0	5082	46	51.1
Assiut Balady 4	90	116	19	174.7	0	1514	56	62.2
Assiut Balady 5	87	0	1	0.0	0	0	-	-
Assiut Balady 6	90	152	23	198.6	0	1649	63	70
Assiut Balady 7	90	88	4	331.0	0	2258	29	32.2
Assiut Balady 8	90	37	2	569.3	0	1813	10	11.1
Assiut Balady 9	90	62	7	246.3	0	955	42	46.7
Assiut Balady 10	90	497	144	138.2	0	3468	81	90
Total	1311						424	32.3

DISCUSSION

An enzyme-linked immunosorbant assay (ELISA) was developed for the serological diagnosis of big liver and spleen disease by Todd *et al.* (1993). The test utilizes specific antigen recovered from the livers of infected hens. This specific antigen is fractionated by gel-filtration chromatography and immobilized on micro-titre plates. Coated plates are produced commercially by Proflock U.S.A. (KPL).

In this work antibodies against a common antigen (P27) was used to detect presence of ALV in tested samples. Results showed great variation among tested flock. The percentage of positive cases was higher in native breed hens and in parent meat type chicks than in egg type chicks. This variation may be attributed to either differences in susceptibility among different breeds or variation on hygienic precautions and biosecurity.

Variation in suspectibility among different breeds or chickens lines were proven by many workers (Fadly and Payne, 2003).

Virus of avian leucosis / sarcoma group seemed to be prevalent in commercial chickens but high rate of these chicks seemed to be carrying endogenous virus. Practically it was of greater importance to detect congenitally transmitting hens than those carrying ALV antigen. The egg albumen was used for detection of exogenous ALV antigen. This method proved to be more efficient in detection of transmission of ALV from dams to their embryos and for shedding ALV into eggs.

Results of ELISA test showed higher titers among meat type breeders with a mean of 127-360 and lower titer in table egg layers with mean titer of 104.

Nearly all examined flocks were carrying ALV antigen in their sera but examination of egg albumin to detect exogenous virus lower incidence and nearly all examined layer flocks were negative while higher incidence was detected in native breeds than in meat type breeders.

Detection of ALV antigen in sera seemed to be correlated with age and small proportion was found in young ages as compared with adult birds. This may be due to exposure after lateral transmission or multiplication of virus in vertically infected chicks.

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